Clinical Pharmacology and Drug Development

Toxicology

Module 2 Topic 5

Toxicology

- **Toxicology** - study of the adverse effects of chemical, physical, or biological agents on people, animals, and the environment.
- It is necessary to prove that a new drug is safe before its first administration to humans.
- ***In vitro toxicology*** studies provide an early indication of the potential for some kinds of toxic effects
- In vitro studies include **Cytotoxicity** studies using cells from higher organisms e.g. liver cells, blood cells etc., **Dermal** or **ocular toxicity** studies, such as Dermal Corrosion, Skin Irritation, and Draize Eye Irritancy

Toxicology

***In vivo* toxicology** methods are used for the following purpose:

- Establish a safe starting dose for clinical studies
- Provide a drug-treatment regimen that would produce the least toxicity
- Assess target organ toxicity and its reversibility
- Provide insight into biomarkers for clinical monitoring

Toxicology

- In general, animal studies are conducted in two species, one **rodent** (e.g., rat, mouse) and one **non-rodent** (e.g. dog, nonhuman primate)
- Other species e.g. rabbits, hamsters, guinea-pigs may be used for special studies, such as vaccine studies
- Effect that is seen both in the rat and in the dog probably involves a common physiological mechanism that is likely to be present in the human

Toxicology

Types of Toxicity studies

- **Safety pharmacology studies** - determine the effects of the drug on specialized organ systems (e.g., cardiovascular, respiratory, neurologic)
- **Acute toxicity studies** – assess the adverse effects of a drug that may result either from a single exposure or from multiple exposures in a short period of time (usually less than 24 hours)

Toxicology

Types of Toxicity studies (contd)

- **Sub-acute toxicity** - show the ability of a toxic substance to cause effects for more than one year but less than the life time of exposed organism
- **Chronic Toxicity/Carcinogenicity** - determine the effects of long-term exposure to the drug, including the ability to produce cancer

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Types of Toxicity studies (contd)

- **Reproductive Toxicity/Teratogenicity** studies evaluate the effects of a drug on reproductive function and ability to produce birth defects
- **Mutagenicity** tests evaluate the likelihood of induction of alterations in the information content (DNA) of an organism or cell that are not due to the normal process of recombination at the time of cell division

Toxicology

Questions that these studies answer

- What are the toxic doses in animals?
- What are the target organs?
- How do the toxic doses compare to the effective/clinical dose(s)?
- Can the toxicities be monitored in patients in the clinical trials?
- Are the toxicities reversible?

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Acute Toxicity Studies:

- An initial step in the assessment of the toxic characteristics of a drug is to use a single dose in each animal for the determination of gross behavior and **LD_{50}** or **median lethal dose**
- LD_{50} value depends on the route of administration. In increasing order - intravenous, intraperitoneal, subcutaneous and oral
- It also helps determine the **therapeutic index** (LD_{50} /ED_{50}), greater the index, safer is the compound

Toxicology

The other values that can be assessed are:

- No-observed-adverse-effect level, **NOAEL**
- Lowest-observed-adverse-effect level, **LOAEL**
- Maximum tolerable concentration, **MTC**
- Maximum tolerable dose, **MTD**
- Minimum lethal concentration, **Lc_{min}**
- Minimum lethal dose, **LD_{min}**

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Subacute Toxicity Studies:

- Examine the adverse effects resulting from repeated exposure over a portion of average lifespan of an experimental animal
- A compound found to be non-toxic in Acute toxicity study may be toxic after prolonged exposure at low doses due to
  - Accumulation
  - Changes in enzyme levels
  - Disruption of physiologic and biochemical homeostasis

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- **Oral** and **inhalation subacute studies** are generally carried out for three months in shorter lived animals (rodents) and 1 year in longer lived animals
- **Dermal studies** are usually performed for 1 month or less

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Chronic Toxicity Studies:

- Assess the ability of a drug to cause harmful effects over an extended period, usually on repeated and continuous exposure
- The result of chronic toxicity study in animals should suggest signs and symptoms of adverse reactions to look for in man

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Carcinogenicity Studies

- Required for clinical administration of over 6+ months
- Purpose is to investigate the potential to cause tumors
- 2 year study in rats and mice (life-time studies, bioassays)
- Usually start around time of Phase III
- Limited study endpoints
  - Palpate for subcutaneous tumor formation
  - Evaluate tissues for microscopic evidence of tumor formation

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Special Toxicity Studies

- Teratogenicity tests
  - Tests for effects on reproduction involve animals, which have been exposed to the test drug from the time of conception to the time they produce their own offspring plus a study of the offspring during growth and development
- Mutagenicity tests
  - Mutagenesis is the induction of alterations in the information content (DNA) of an organism or cell that is not due to the normal process of recombination. This change may occur in germ cells or somatic cells

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Mutagenicity tests (contd)

Types of Mutation

- **Point mutations**- Alteration in a single nucleotide pair in the DNA molecule viz. A-T or G-C
- **Chromosome aberrations**- breaks and rearrangement of chromosomes

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Mutagenicity tests (contd)

- ***In vitro* studies** use bacteria e.g. Salmonella typhimurium for mutations at G-C pairs or Escherichia coli for mutations at A-T pairs
- **Animal studies** using rats or mice can indicate genetic damage in the form of structural or numerical chromosome aberrations
- **Host Mediated Assay** helps detect substances which are not mutagenic *in vitro* but are converted to active mutagens in mammals OR substances may be mutagenic *In-vitro* but get detoxified by mammalian system

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Toxicology Study Durations

Duration of Clinical Trial	Minimum Duration of Repeat Dose Toxicity Studies	
	Rodents	**Non-Rodents**
Single dose	2-4 Weeks	2 Weeks
Up to 2 Weeks	2-4 Weeks	2 Weeks
Up to 1 Month	1 Month	1 Month
Up to 3 Months	3 Months	3 Months
Up to 6 Months	6 Months	6 Months
> 6 Months	9 Months	9 Months

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Animal Model Requirements in Toxicity Studies

Study Type	Minimum No. of Animals Required		Dosing	Age at Start of Study
	Males	Females		
Acute oral (rat), dermal, or inhalation (rat)	5	5	Single	Young adult
Eye and skin irritation (rabbit)	6[a]		Single	Young adult
Dermal sensitization (guinea pig)	[b]		Repeated	Young adult
21-Day dermal (rat, rabbit, or guinea pig)	5	5	Repeated	Rat, 200–300 g; rabbit, 2.0–3.0 kg; guinea pig, 350–450 g
90-Day oral (rat)	10	10	Repeated	6–8 weeks
90-Day inhalation (rat)	10	10	Repeated	Young adult
90-Day dermal (rat, rabbit, or guinea pig)	10	10	Repeated	Rat, 200–300 g; rabbit, 2.0–3.0 kg; guinea pig, 350–450 g
90-Day or chronic (1 year) oral (dog)	4	4	Repeated	4–6 months
Reproduction (rat)[c]	20	20	Repeated	8 weeks
Teratology				
Rat		20[d]	Repeated	Young adult
Rabbit		12[d]	Repeated	Young adult
Chronic toxicity (1 or 2 year) (rat)	20	20	Repeated	6–8 weeks
Oncogenicity (lifetime) (rat and mouse)	50[e]	50[e]	Repeated	6–8 weeks

[a] Either males or females may be used in this test.
[b] The number of animals used depends on the method used. Several different experimental methods are acceptable.
[c] EPA prefers that one male rat be housed with one female during mating.
[d] Number of pregnant females required.
[e] 50 rats and 50 mice of each sex